

## FLAVONOID CHEMISTRY AND EVOLUTION OF *GUNNERA* (GUNNERACEAE) IN THE JUAN FERNANDEZ ISLANDS, CHILE

### QUIMICA DE FLAVONOIDES Y EVOLUCION DE *GUNNERA* (GUNNERACEAE) EN EL ARCHIPIELAGO DE JUAN FERNANDEZ, CHILE

Patricia Pacheco<sup>\*\*\*</sup>, Daniel J. Crawford<sup>\*</sup>  
 Tod F. Stuessy<sup>\*</sup> and Mario Silva O.<sup>\*\*</sup>

#### ABSTRACT

*Gunnera* in the Juan Fernandez Islands consists of the endemic species *G. bracteata*, *G. masafuerae* and *G. peltata*. Examination of vegetative and reproductive features of continental species of Section *Panke* suggests that *G. tinctoria* of the mainland is the likely progenitor for the islands endemics. Reconstruction of phylogeny via cladistic analysis reveals that an early line of divergence occurred on the older island, Masatierra, resulting in *G. bracteata* and *G. peltata*. Propagules from the latter species dispersed to the younger island, Masafuera, and speciated in isolation as *G. masafuerae*. New chromosome counts from endemic island species as well as from *G. tinctoria* are all  $n = 17$ , showing no change in chromosome number during evolution of the complex in the archipelago. Fifteen flavonoid compounds isolated from leaves of these taxa, plus from five additional species from Bolivia, México and Perú, are primarily glycosides of quercetin, kaempferol and isorhamnetin. Both gains and losses of compounds have occurred during evolution of the group.

KEYWORDS: *Gunnera*, insular evolution, flavonoids, Juan Fernández Archipiélago.

#### RESUMEN

El género *Gunnera* en el Archipiélago de Juan Fernández está representado por tres especies endémicas: *G. bracteata*, *G. masafuerae* y *G. peltata*. El examen de los caracteres vegetativos y reproductivos de especies continentales de la sección *Panke* sugieren que *G. tinctoria* de Chile continental es la posible progenitora de las especies endémicas insulares. La filogenia del grupo insular, reconstruida por medio de análisis cladístico revela que *G. bracteata* y *G. peltata* se originaron por una divergencia temprana ocurrida en Masatierra, la isla más antigua. *Gunnera masafuerae* resultó de la especiación en aislamiento de propágulos de *G. peltata* que se dispersaron a Masafuera, la isla más joven. Nuevos recuentos de cromosomas de *G. tinctoria* y de las tres especies endémicas resultaron ser todos  $n = 17$ , demostrando que no ha habido cambios en número de cromosomas durante la evolución del grupo en el archipiélago. De las especies endémicas y de cinco especies de Bolivia, México y Perú se aislaron quince compuestos flavonoides. Estos son principalmente glicósidos de quercetina, kaempferol e isorhamnetina. Durante la evolución de *Gunnera* en el archipiélago han ocurrido ganancias y pérdidas de flavonoides.

PALABRAS CLAVES: *Gunnera*, evolución insular, flavonoides, Archipiélago Juan Fernández.

#### INTRODUCTION

Evolutionary phenomena in flowering plants are sometimes more easily investigated on oceanic islands than in continental areas. These small isolated areas have been the sites of dramatic evolutionary differentiation and adaptations.

\* Department of Plant Biology, The Ohio State University, 1735 Neil Avenue, Columbus, Ohio, 43210.

\*\* Departamento de Botánica, Universidad de Concepción, Concepción, Chile.

\*\*\* Present address: Departamento de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Oceanic islands contain unique plants, often quite different from close mainland relatives, which have diverged into different ecological niches in a restricted geographical area. Many evolutionary investigations have been made on island taxa (e.g., Carlquist, 1965, 1974; Carr and Kyhos, 1981; Crawford, Stuessy and Silva, 1986, 1988; Crins, Bohm and Carr, 1988; Gardner, 1976; Pacheco, Crawford, Stuessy and Silva, 1985; Patterson, 1984; Sanders, Stuessy, Marticorena and Silva, 1987; Witter and Carr, 1988). These numerous studies have established the potential of understanding phylogeny and evolutionary processes in island biotas.

The Juan Fernández Archipelago is an example of oceanic islands with a high degree of endemism in the native flora. The archipelago (Fig. 1) is located in the Pacific Ocean about 670 km west of mainland Chile at 33° S latitude. There are three major islands: Masatierra (=Isla Robinson Crusoe), Masafuera (=Isla Alejandro Selkirk) and Santa Clara just off the coast of Masatierra. Masatierra and Masafuera are separated by 180 km of ocean in an East-West orientation. The ages of these islands are known to be 3,7-4,2 my for Masatierra and 1-2 my for Masafuera (Stuessy, Foland, Sutter, Sanders and Silva, 1984). In these geographically isolated islands there has evolved a unique flora characterized by a high degree of endemism. Of a total of 147 native species, 69% are endemic (Skottsberg, 1921).

The Flora of the Juan Fernández Islands has been the object of several studies. Floristic studies have been published by Johow (1896), Philippi (1856), and Skottsberg (1921, 1951, 1956). More recently the emphasis has change to evolutionary studies and the endemic taxa have been analyzed in terms of flavonoid evolution (Pacheco *et al.*, 1985; Crawford *et al.*, 1986), allozyme variation (Crawford, Stuessy and Silva 1987, 1988), chromosome numbers (Sanders, Stuessy and Rodríguez, 1983; Spooner, Stuessy, Crawford and Silva, 1987; Sun, Stuessy and Crawford, 1990), and phylogeny and patterns of speciation (Lammers, Stuessy, and Silva 1986; Sanders *et al.*, 1987; Crawford, Whitkus and Stuessy, 1987; Stuessy, Crawford and Marticorena, 1990).

Among the taxa of the Juan Fernández Islands, the endemic species of *Gunnera* (Gunneraceae) are particularly interesting

because they are the largest herbs in the archipelago, they have speciated within the islands, they belong to a genus with ample distribution on mainland South America, and they represent the only known case of natural interspecific hybridization between two species growing on Masatierra (Skottsberg, 1921; Pacheco, Stuessy, and Crawford, 1991).

On a worldwide basis *Gunnera* (Gunneraceae) has been segregated into six subgenera based on morphological distinctions (Schindler, 1905; Bader, 1961): *Gunnera*, *Milligania*, *Ostenigunnera*, *Panke*, *Perpensum*, and *Pseudogunnera*. The endemic species of *Gunnera* of the Juan Fernández Archipelago belong to the subgenus *Panke*, which is the largest group in *Gunnera*, with approximately 50 species distributed in South America and Hawaii (Mora-Osejo, 1984, 1970; St. John, 1946, 1957). The species in subgenus *Panke* are gigantic perennial herbs with creeping of suberect rhizomes. The leaves, enormous and long-petiolated, are in rosette at the end of the rhizome. In the terminal bud the leaf primordium is protected by scales (modified leaves, or "lepidofilos", Mora-Osejo, 1984). The inflorescence, a large compound spike, bears numerous small red to brownish drupes. The habitats in which species of the subgenus *Panke* are found are characterized by moderate to heavy rainfall and moderate to high elevation (Bader, 1961). In the Juan Fernández archipelago, subgenus *Panke* is represented by three endemic species: *Gunnera bracteata* Steud., *G. masafuerae* Skottsberg, and *G. peltata* Phil.

The purposes of the present study are to: 1) determine the relationships of the endemic Juan Fernández species of *Gunnera* with their closest mainland relatives; 2) determine the phylogenetic affinities of the endemic species in the Juan Fernández Islands; 3) examine the flavonoid components of the endemic species of *Gunnera* and their continental relatives; and 4) interpret all the data for evolutionary implications, with particular reference to changes in the flavonoid system during evolution of *Gunnera* in the archipelago.

## MATERIALS AND METHODS

**Morphological analysis.** Three endemic

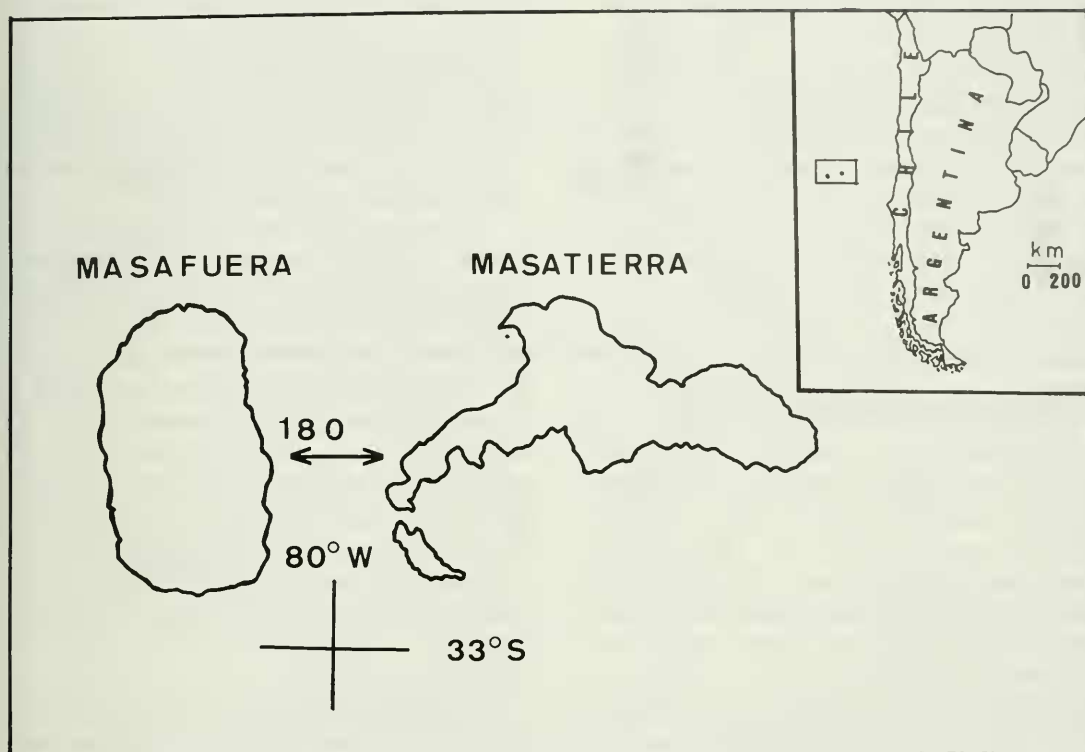


FIG 1. Map showing the location of the Juan Fernández Archipiélago in southern South America.

species were collected during expeditions to the Juan Fernández Islands organized by the Department of Botany of The Ohio State University and the University of Concepción in 1980, 1981, 1984 and 1986. Voucher specimens are at CONC and OS. The study of the relationships of the islands species with continental species in *Gunnera* subgenus *Panke* was based also on material collected during the expeditions to mainland Chile (1986), Bolivia (1987) and Perú (1987), and on material borrowed from the following herbaria: F, MO, NY and UC. Based on these morphological studies, *G. tinctoria* was judged the closest continental relative. For phylogenetic reconstruction among the endemic island species, *G. tinctoria* was selected as the outgroup. Eight vegetative characters were used (Table I), and the Hennigian argumentation method (maximum parsimony) was the manual algorithm of choice (Kavanaugh 1972). Reproductive features were not used here because of limited representation in available

herbarium material. Character state networks were polarized by the outgroup criterion (Crisci and Stuessy, 1980). The basic data matrix is given in Table II.

**Flavonoid analysis.** For flavonoid analysis, ground leaf material was extracted with 85% and 50% methanol. The extracts were taken to dryness, suspended in water, filtered, and then applied to 55 x 35 cm 3MM chromatographic paper. The two-dimensional chromatograms (2-D) were developed inter-butanol: acetic acid:water (3:1:1; TBA) in the first direction and in 15% acetic acid (15% HOAc) in the second direction. Spots on the chromatograms representing individual compounds were eluted with spectral grade methanol. When purification of individual flavonoids was necessary, this was accomplished by thin layer chromatography (TLC) on precoated polyamide DC-6 plates (Macherey-Nagel). The solvent system used was 1,2 dichloroethane: methanol: methyl ethyl ketone: water: formic acid (50: 25: 20.5: 4: 0.5).

Identifications of the flavonoids were done by the study of a set of six ultraviolet spectra obtained following standard procedures (Mabry, Markham and Thomas, 1970). Flavonoid glycosides were hydrolyzed with 0.1N trifluoroacetic acid in screw-cap test tubes in water bath for one hour (Wilkins and Bohm, 1976). Sugars were identified by circular co-chromatography with standards on precoated cellulose thin layer plates (Macherey-Nagel) using as solvent system pyridine: ethyl acetate: water (6:3:2) (Becker, Exner and Averett, 1977). The chromatograms were sprayed with p-anisidine phthalate (1.0 g phthalic acid and 1.0 ml anisidine in 100 ml of 96% ethanol) to visualize the sugar compounds.

**Chromosome counts.** Living material of *Gunnera bracteata*, *G. peltata*, and *G. masafuer-ae* was collected during the expedition to Juan Fernández Islands in January and February of 1984. *Gunnera tinctoria* was collected near Concepción, Chile in February, 1984. For mitotic studies, root tips of the four species were treated with 0.2% colchicine, fixed in 95% ethanol: 99% propionic acid (4:1) for two days, stained with acetocarmine, macerated with 45% acetic acid at 60, and then squashed. Material for meiotic counts was obtained from *G. boliviana* in 1987. Buds were fixed in Carnoy's solution and chromosomes were counted following the conventional squash techniques with acetocarmine (Snow, 1963).

## RESULTS

**Phylogeny.** A cladogram of phylogenetic relationships is shown in Fig. 2. *Gunnera peltata* is the most primitive species of the group in the archipelago. It is endemic on Masatierra, and it grows throughout the forest in humid valleys from 350-500 m. The leaves are peltate, soft-chartaceous, and the fruits are small drupes. The habitat of *G. peltata* is very similar to that of *G. tinctoria* of mainland Chile. *Gunnera masafuer-ae* is closely related to *G. peltata*. It evolved on the younger island, Masafuera, where it grows along the water courses and on walls of the canyons, similar to the habitats of *G. peltata*. It is also abundant in fog-swept highlands especially along water courses and depressions in fern beds. *Gunnera masafuer-ae* differs from *G. peltata* in

having reniform leaves and reaching 1,100 m elevations. *Gunnera bracteata* is the most divergent species in the group. This species, endemic to Masatierra, is confined to the ridges above 400 m. The leaves are completely glabrous and smaller than those of *G. peltata* and *G. masafuer-ae*. *Gunnera bracteata* is adapted to more open habitats outside the range of the forest vegetation on ridges exposed to continuous winds.

**Cytology.** Chromosome numbers for species of *Gunnera* subgenus *Panke* have been reported to be  $2n = 34$  (Dawson, 1983; Mora-Osejo, 1984). Our new counts (Table III) for the three endemic species of *Gunnera*,  $2n = 34$ , suggest that the endemic species are polyploids at the tetraploid level and that speciation within the archipelago has occurred without change in ploidy level. The same chromosome number of  $2n = 34$  in *G. tinctoria*, the presumed ancestor of the endemic group, indicated that there have been no changes in ploidy level relative to the mainland ancestor. The endemic species of *Gunnera*, therefore, can be regarded as ancient polyploids (Sanders *et al.*, 1983).

**Flavonoid analysis.** Fifteen flavonoids were isolated from the species of *Gunnera* studied (Table IV). Their identities are: 1, Quercetin 3-0 arabinoside; 2, Quercetin 3-0 glucogalactoside; 3, Quercetin 3-0 digalactoside; 4, Quercetin 3-0 diglucoside; 5, Quercetin 3-0 xyloxyglucoside; 6, Quercetin 3,7-0 diglucoside; 7, 8, and 9, Kaempferol 3-0 glycosides; 10, Kaempferol; 11, Isorhamnetin 3-0 glucoside; 12, Isorhamnetin; 13, Flavone glycoside; 14, 15, unknown phenolics. The flavonoid pattern in general is characterized by the presence of glycosides of quercetin, kaempferol and isorhamnetin. Kaempferol glycosides are common in species of *Gunnera* from Bolivia, Perú and México, but they are absent from island endemic species and from *G. tinctoria*. Isorhamnetin is restricted to the endemic species of *Gunnera* and *G. tinctoria*; however, a trace might also be present in *G. bolivari*.

## DISCUSSION

**Origin of *Gunnera* in the Juan Fernández Islands.** The founder population of *Gunnera* must have arrived in the islands within the last

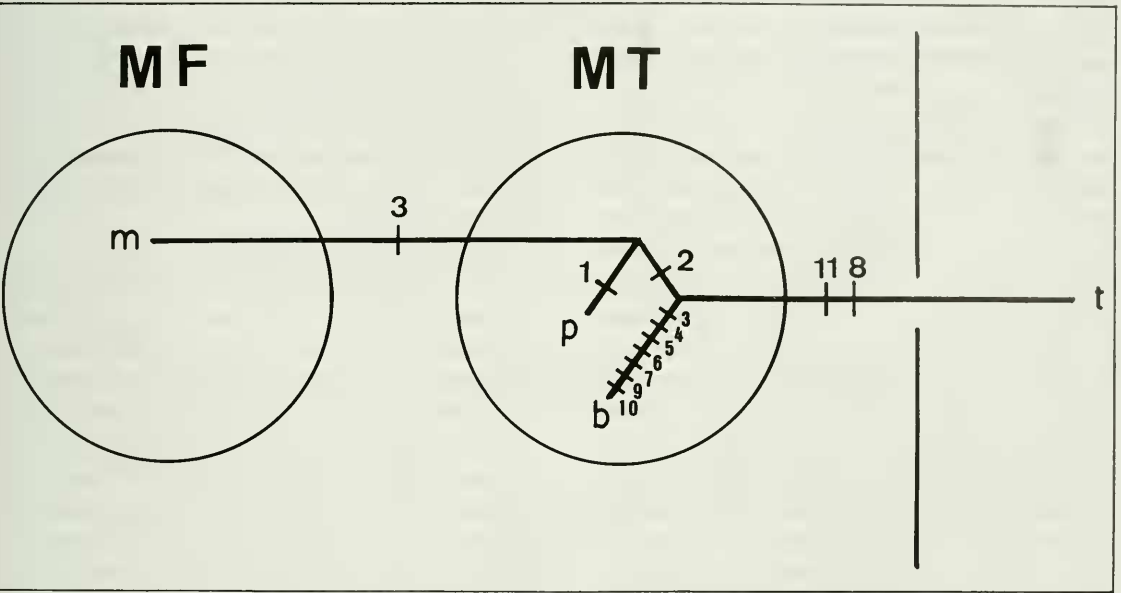


FIG 2. Cladogram showing evolutionary relationships among endemic of *Gunnera* from the Juan Fernández Islands and their presumed ancestor, *G tinctoria*, from mainland Chile. Characters and states are given in Table I. MF = Masafuera; MT = Masatierra; b = *G. bracteata*; m = *G. masafuerar*; p = *G. peltata*; t = *G. tinctoria*.

four million years, the age calculated for Masatierra (Stuessy *et al.*, 1984). *Gunnera* is a small group in the islands which suggests that arrival may have occurred somewhat late during the colonization process of the archipelago, when the ecological opportunities were not as numerous as in the earlier phases. Colonization of Masafuera must have occurred within the last 1 to 2 million years via propagules from Masatierra.

It seems likely that *Gunnera* in the Juan Fernández Archipiélago originated from a single introduction, and is therefore monophyletic. The geographical isolation of the archipiélago, the probabilities of the colonization process, the chemical homogeneity of the group, and the natural hybridization of the two endemic species on Masatierra (Pacheco *et al.*, 1991) all support the idea of a single introduction. Analysis of morphological characters in species from South America plus study of species elsewhere in the

genus reveal that *G. tinctoria*, a widely distributed species in continental Chile, is clearly the closest relative of the endemic species of *Gunnera* on the islands. Further support for this conclusion comes from the flavonoid contents of selected species of *Gunnera* of subgenus *Panke* from South America (Table IV). All the species of *Gunnera* from Bolivia, Perú and México are characterized by the presence of kaempferol and its glycosides, compounds which are absent in the endemic species of *Gunnera* as well as in *G. tinctoria*. *Gunnera tinctoria* and the endemic species on the islands form one group characterized by the presence of a isorhamnetin (flavonoid 12) which is absent from the group of *Gunnera* from Bolivia, Perú and México. The morphological and flavonoid data suggest that *Gunnera tinctoria* is the closest relative of the endemic species in the Juan Fernández Archipelago, and it was therefore chosen as the outgroup for the cladistic analysis. Since the continental area is older than

the islands, it is highly probable that the most closely related taxon on the mainland would represent the ancestor of the insular taxa, rather than the reverse.

Propagules of *G. tinctoria*, or a common ancestor of *G. tinctoria* and the endemic island species, probably came from Southern South America. The fruits of *Gunnera* are small fleshy drupes that might well be adapted for dispersal by frugivorous birds (Carlquist, 1974). Seeds of *Gunnera* from mainland Chile could have arrived inside the digestive system of birds on erratic courses brought by cyclonic winds. There are no known usual migration routes between Southern South America and the Juan Fernández Archipelago (Dorst, 1961), but birds from mainland Chile, especially waterfowl, have been recorded occasionally on the Juan Fernández Islands (Weller, 1980). Dispersal from Masatierra to Masafuera was probably effected by internal transport by endemic birds. The Juan Fernández Petrel and Masafuera Petrel are the two possible vectors for dispersal of *Gunnera* fruits. Although they nest only in Masafuera, they make long flights out into the ocean and it is likely that they make occasional trips to Masatierra (Johnson, 1965).

The considerable divergence in morphological characters of *G. bracteata* led us to test the hypothesis of a double introduction of this group to the islands. Two species have been proposed as possible close relatives of *G. bracteata*: *G. bolivari* and *G. magnifica*. St. John (1957) described *G. magnifica* from Colombia and suggested that its closest relative was *G. bracteata*. We dismiss this idea based on the shape and the size of the scales and leaf vestiture. In *G. magnifica* the scales are 10-39 cm long and lance-linear with the margin lacinate. In *G. bracteata* the scales are entire, broad-ovate and 2.5 to 4 cm long. The petioles and blades are muricated and covered with unicellular hairs in *G. magnifica* whereas in *G. bracteata* the leaves are completely glabrous. According to MacBride (1959), *G. bolivari* could be another close relative of *G. bracteata*. He based this statement on the size of the leaves, which in these two species are smaller than in many other species of *Gunnera*, and also on their smooth and glabrous conditions. We agree that these three characters are also present in *G. bracteata* but while *G. bolivari* is almost ebracteate, *G. bracteata* presents conspicuous

spatulate bracts in the inflorescence. Additional support for dismissing a close relationship between *G. bracteata* and *G. bolivari* comes from the analysis of the flavonoid compounds of these two species. In *G. bolivari* the flavonoid profile is characterized by the presence of kaempferol glycosides and the apparent absence of isorhamnetin. This same flavonoid pattern is also shown by species from Perú, Bolivia and México (Table IV), but not in *G. bracteata*. The profile of the latter species is clearly most similar to those of the other two endemic species and to that of *G. tinctoria*.

**Flavonoid evolution.** To investigate flavonoid changes during the evolution of the endemic species of *Gunnera* in the Juan Fernández Islands, we assume that the relationships based on morphological data, and depicted as a cladogram (Fig. 2), reflect the actual evolutionary relationships among the three endemic taxa and their presumed ancestor, *G. tinctoria*. Evolution of the flavonoid system can be seen by superimposing the distribution of flavonoids (Table IV) upon the hypothetical evolutionary relationships (Fig. 3). The evolution of the group on the islands is characterized by both loss and gain of compounds. Changes in the flavonoid system during speciation of the group on the islands are characterized by two main features. First, little flavonoid divergence has accumulated between the island species and their presumed progenitor on the mainland. The flavonoid system in the endemic species is characterized by the loss of isorhamnetin 3-O-glucoside and the gain of quercetin 3-O-xyloxyglucoside. Second, minimal changes in flavonoid chemistry have occurred during speciation among the endemic species on the archipelago, and these are largely losses of compounds. Quercetin 3-O-digalactoside (compound 3) is lost in the lines leading to *G. peltata* and *G. bracteata*. An additional loss occurred in *G. bracteata* where quercetin 3-O-xyloxyglucoside has been lost. One innovation that occurred during the evolution of *G. masafuerae* in the younger island was the appearance of the flavonol (compound 6) quercetin 3,7-O-digalactoside. This gain mutation must have occurred within 1-2 my, as this is the age of Masafuera. Simple flavonoid profiles in island endemics have been documented previously (Gardner, 1976; Patterson, 1984). Explanation for reduction in number of flavonoid is based

TABLE I. Characters and states used in the cladistic analysis of endemic species of *Gunnera* from the Juan Fernández Islands and *G. tinctoria* from mainland Chile.

Character	Character state	
	Primitive (0)	Derived (1)
<b>Leaf blade</b>		
1. base	cordate	peltate
2. lobing	1/4 or more of blade	less than 1/4 of blade
3. lobe apex	acute	obtuse
4. undersurface vesture	pilose	glabrous
5. glandularity (verrucate)	present	absent
<b>Scale</b>		
6. shape	lance-linear	ovate
7. margin	lacerate	entire
<b>Inflorescence</b>		
8. branch shape	short (3-5 cm) and squat	long (6-9 cm) and thin
9. branch vesture	strigose	glabrous
10. bract shape	linear	spathulate
11. fruit surface (dry)	conspicuously wrinkled (subreticulate)	scarcely to moderately wrinkled

 TABLE II. Basic data matrix of states of characters in species of *Gunnera* from the Juan Fernández Island and *G. tinctoria* from mainland Chile. See Table I for descriptors and numerical assignments of characters and states.

Taxa	Characters										
	1	2	3	4	5	6	7	8	9	10	11
<i>G. bracteata</i>	0	0	1	1	1	1	1	1	1	1	1
<i>G. masafuerae</i>	0	1	1	0	0	0	0	1	0	0	1
<i>G. peltata</i>	1	1	0	0	0	0	0	1	0	0	1
<i>G. tinctoria</i>	0	0	0	0	0	0	0	0	0	0	0

TABLE III. New chromosome number of species of *Gunnera* subgenus *Panke* from the Juan Fernández Islands and two species from mainland South America. Vouchers deposited at OS.

Taxon	Chromosome Number	Voucher
<i>G. boliviana</i> Morong	n = 17*	BOLIVIA: COCHABAMBA: Villa Tunari, Pacheco <i>et al.</i> 1563.
<i>G. bracteata</i> Steud. ex Benn.	2n = 34*	MASATIERRA: Quebrada Villagra Stuessy <i>et al.</i> 6481-A
<i>G. masafuerae</i> Skottsbo.	2n = 34*	MASAFUERA: Quebrada Las Casas, Pacheco and Ruiz 6398
<i>G. peltata</i> Phil.	2n = 34*	MASATIERRA: Puerto Francés, Pacheco and Ruiz 6670
<i>G. tinctoria</i> (Mol.) Mirb.	2n = 34	CHILE: CONCEPCION: San Pedro, near Concepción, Stuessy <i>et al.</i> 6692

\* First report for the taxon

TABLE IV. Distribution of flavonoids present in the three endemic species of *Gunnera* in the Juan Fernández Islands, *G. tinctoria*, and other selected species of *Gunnera* from South America

Species and Collection number	Locality	Flavonol										Fla		Unk			
		Q - gly						K - gly				K		I - glu I		F - g ly	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
<b>Endemic species</b>																	
<i>G. bracteata</i>																	
6481-21A	MT: Q. Villagra	x	x													x	x
6481-22A	" "	x	x		x											x	x
6481-24	" "	x	x		x							x	x	x	x	x	x
6481-28	" "	x	x		x							x	x	x	x	x	x
6481-32	" "	x	x		x							x	x	x	x	x	x
6481-33	" "	x	x		x							x		x	x	x	x
6494-25	" "	x	x		x											x	x
<i>G. masafuerae</i>																	
5050	MF: Q. Las Casas	x	x	x	x	x						x	x	x	x	x	x
6362	MF: Q. Mono	x	x	x	x	x	x					x	x	x	x	x	x
6396	MF: Q. Las Casas	x	x	x	x	x						x	x	x	?	x	
8017	MF: " "	x	x	x	x	x	x					x	x	x	x	x	x
8081	MF: " "	x	x	x	x	x	x					x	x	x	x	x	x



TABLE IV. Continued

Species and Collection number	Locality	Flavonol										Fla				
		1	2	Q - gly		5	6	K - gly			K	I - glu I		F - gly	Unk	
				3	4			7	8	9	10	11	12	13	14	15
<i>G. peltata</i>																
6481-65	MT: Q. Villagra	x	x		x	x							x	x	x	x
6671-68	MT: Pto. Francés	x	x		x	x							x	x	x	x
6671-69	MT: " "	x	x		x	x								x		x
6671-71	MT: " "	x	x		x								x	x	x	x
6671-75	MT: " "	x	x		x	x							x	x	x	x
Mainland species																
<i>G. tinctoria</i>																
1090	CHILE: Malleco	x	x	x								x	x	x	x	x
1101	CHILE: Malleco	x	x	x	x							x	x	x	x	x
1178	CHILE: Chiloé	x	x	x	x							x	x	x	x	x
1207	CHILE: Chiloé	x	x	x	x							x	x	x	x	x
1247	CHILE: Ñuble	x	x		x							x	?	x	x	x
1304	CHILE: Concepción	x	x		x							x		x	x	x
1917	CHILE: Arauco	x	x	x	x							x	x	x	x	x
6692	CHILE: Concepción	x	x	x	x							x	?	x	x	x
6786	ARGENTINA: Río Negro	x	x	x								x	x	x	x	x
<i>G. bolivari</i>																
1421	PERU: Pillahuata	x	x		x			x	x				x	?	x	x
<i>G. boliviana</i>																
1563	BOLIVIA: Cochab.	x	x						x						x	x
<i>G. margaretae</i>																
1547	BOLIVIA: Cochab.	x	x					x	x	x					x	x
<i>G. peruviana</i>																
1411	PERU: Paucartambo	x	x		x			x	x	x					x	x
<i>G. mexicana</i>																
2793	MEXICO: Chiapas	x	x		x			x	x	x	x				x	x

Fla = Flavone; glu = glucose; gly = arabinose, galactose, glucose, xylose; I = isorhamnetin; K = kaempferol; Q = quercetin; Unk = unknown.

Collection numbers: 1000s are Pacheco *et al.*, *G. mexicana* is Spooner 2793; 5000s, 6000s and 8000s are Stuessy *et al.*

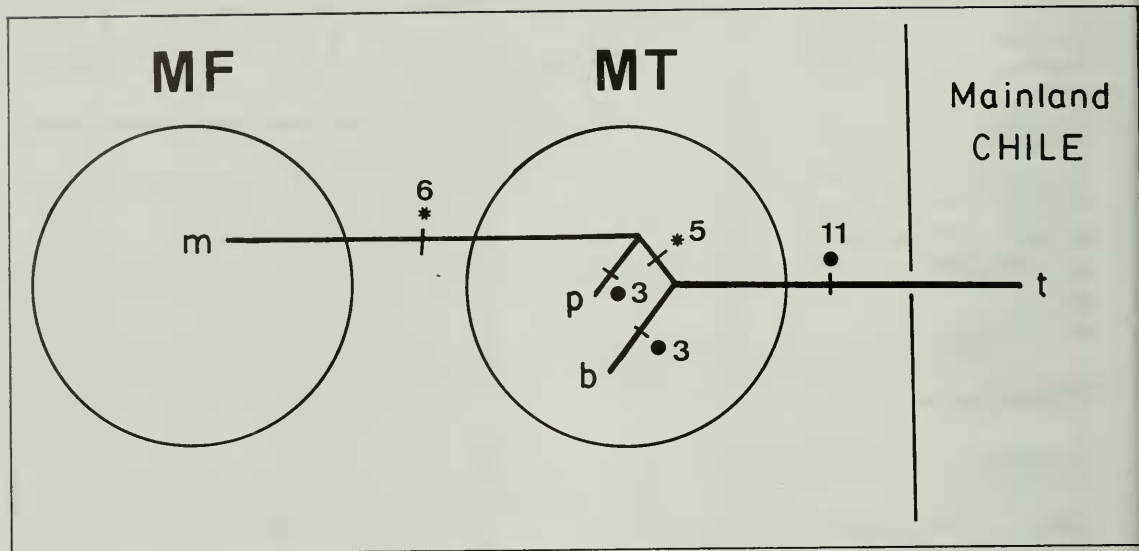


FIG. 3. Changes in the flavonoid system in species of *Gunnera* from the Juan Fernández Islands, superimposed on a hypothetical evolutionary tree. Same labels as in Fig. 2. Numbers refer to flavonoid compounds: 3 = quercetin 3-O-digalactoside, 5 = quercetin 3-O-xyloxyl glucoside, 6 = quercetin 3, 7-O-diglucoside, 11 = isorhamnetin 3-O-glucoside. \* = gain of compounds, 0 = loss.

largely on the suspected role of these compounds as defensive mechanisms against predators or pathogens (Levin, 1971; Gardner, 1976), which are often not present in at least the same intensity in islands. Recent results by Ganders, Bohm and McCormick (1990) for *Bidens* in the Hawaiian Islands, however, cast doubt on this hypothesis.

**Speciation in the endemic species of *Gunnera*.** The mode of speciation of *G. masafuerae* can be postulated as largely geographical. *Gunnera masafuerae* evolved on Masafuera in isolation from populations on Masatierra within 1-2 my, the age calculated for this island (Stuessy *et al.*, 1984). On Masatierra, *G. bracteata* probably evolved on the highest ridges. It is known that geographical isolation can occur over short distances in an insular environment (Carlquist, 1974).

Speciation in *Gunnera* on Masatierra has not been accompanied by complete reproductive isolation, as they are now hybridizing extensively in Valle Villagra on Masatierra. Lack of postzygotic reproductive isolation and minimal genetic divergence are characteristics that have been reported for insular taxa (Gillet and Lim, 1970;

Carlquist, 1974; Carr and Kyhos 1981; Crawford *et al.*, 1987). In *Gunnera*, speciation on Masatierra has involved conspicuous morphological changes but little genetic isolation, and as a result, *G. bracteata* and *G. peltata* hybridize in zones of contact. These two species have come in contact probably due to the reduction in total surface area of the island (Sanders *et al.*, 1987) and by human disturbance of the habitats (e. g., building of trails). Hybridization in the disturbed area in Quebrada Villagra is one of the few examples of extensive hybridization in an island environment and the only example of natural hybridization in the flora of Juan Fernández Islands (Pacheco *et al.*, 1991).

#### ACKNOWLEDGEMENTS

This represent part of a dissertation in partial fulfillment of the Ph. D. degree in the Department of Plant Biology at The Ohio State University. Thanks go to Michael L. Evans for

critically reading the thesis manuscript; NSF for support under grants INT-8317088 and BSR-8306436; OEA for a PRA Fellowship; the Tinker Foundation for a travel award; OSU for an Alumni Research Award; Sigma Xi for a Grant-in-Aid; and CONAF of Chile for permission to collect in the Robinson Crusoe National Park.

## LITERATURE CITED

- BADER, F. J. W. 1961. Das areal der Gattung *Gunnera* L. Bot. Jahrb. Syst. 90: 281-293.
- BECKER, H., J. EXNER and J. E. AVERETT. 1977. Circular chromatography, a convenient method for phytochemical analyses. Phytochem. Bull. 10: 36-41.
- CARLQUIST, S. 1965. Island life: A natural history of the islands of the world. The Natural History Press, Garden City, New York.
- CARLQUIST, S. 1974. Island Biology. Columbia Univ. Press, New York.
- CARR, G. D. and D. W. KYHOS. 1981. Adaptive radiation in the Hawaiian silversword alliance (Compositae-Madiinae). Cytogenetics of spontaneous hybrids. Evolution 35: 543-556.
- CRAWFORD, D. J., T. F. STUESSY and M. SILVA O. 1986. Leaf flavonoid chemistry and the relationships of the Lactoridaceae. Pl. Syst. Evol. 153: 133-139.
- CRAWFORD, D. J., T. F. STUESSY and M. SILVA O. 1987. Allozyme divergence and the evolution of *Dendroseris* (Compositae:Lactuceae) on the Juan Fernández Islands. Syst. Bot. 12: 435-443.
- CRAWFORD, D. J., R. WHITKUS and T. F. STUESSY. 1987. Plant evolution and speciation on oceanic islands. In: M. Urbanska (ed. ), Differentiation Patterns in Higher Plants, pp. 183-199. Academic Press, London.
- CRAWFORD, D. J., T. F. STUESSY and M. SILVA O. 1988. Allozyme variation in *Chenopodium sancta-clarae*, an endemic species of the Juan Fernández Islands, Chile. Biochem. Syst. Ecol. 16: 279-284.
- CRINS, W. J., B. A. BOHM, and G. D. CARR. 1988. Flavonoids as indicators of hybridization in a mixed population of lava-colonizing Hawaiian tarweeds (Asteraceae: Heliantheae: Madiinae). Syst. Bot. 13: 567-571.
- CRISCI, J. V. and T. F. STUESSY. 1980. Determining primitive character states for phylogenetic reconstruction. Syst. Bot. 5: 112-135.
- DAWSON, M. I. 1983. Chromosome numbers of three South American species of *Gunnera* (Gunneraceae). New Zealand J. Bot. 21: 457-459.
- DORST, J. 1961. The Migration of Birds. The Riverside Press: Cambridge, Mass.
- GANDERS, F. R., B. A. BOHM and S. P. MCCORMICK. 1990. Flavonoid variation in Hawaiian *Bidens*. Syst. Bot. 15: 231-239.
- GARDNER, R. C. 1976. Evolution and adaptive radiation in *Lipochaeta* (Compositae) of the Hawaiian Islands. Syst. Bot. 1: 383-391.
- GILLETT, G. W. and E. K. S. LIM. 1970. An experimental study of the genus *Bidens* (Asteraceae) in the Hawaiian Islands. Univ. Calif. Pub. Bot. 56: 1-63.
- JOHNSON, A. W. 1965. The birds of Chile and adjacent regions of Argentina, Bolivia and Perú. I. Platt Establecimientos Gráficos, Buenos Aires, 397 pp.
- JOHOW, F. 1896. Estudios sobre la Flora de las Islas de Juan Fernández, Santiago, XI, 289 pp; 18 lám, 2 mapas.
- KAVANAUGH, D. H. 1972. Hennig's principles and methods of phylogenetic systematics. Biologist 54: 115-127.
- LAMMERS, T. G., T. F. STUESSY and M. SILVA O. 1986. Systematic relationships of the Lactoridaceae, an endemic family of the Juan Fernández Islands, Chile. Pl. Syst. Evol. 152: 243-266.
- LEVIN, D. A. 1971. Plant Phenolics: an ecological perspective. Amer. Naturalist 105: 157-181.
- MABRY, T. J., K. R. MARKHAM and M. S. THOMAS. 1970. The Systematic Identification of Flavonoids. Springer-Verlag, Berlin. 354 pp.
- MACBRIDE, J. F. 1959. Flora of Peru. Field Mus., Bot. Ser. 12 (pt. 5, Nº 1): 3-8.
- MORA-OSEJO, L. E. 1970. Nuevas especies de *Gunnera* del Neotrópico. Mutisia 45: 1-11.
- MORA-OSEJO, L. E. 1984. Haloragaceae. Flora de Colombia. Monografía 3, pp. 1-178. Fac. Ciencias de la Universidad de Colombia, Bogotá.
- PACHECO, P., D. J. CRAWFORD, T. F. STUESSY and M. SILVA O. 1985. Flavonoid evolution in *Robinsonia* (Compositae) of the Juan Fernández Islands. Amer. J. Bot. 72: 989-998.
- PACHECO, P., T. F. STUESSY and D. J. CRAWFORD. 1991. Natural interspecific hybridization in *Gunnera* (Gunneraceae) of the Juan Fernández Islands, Chile. Pacific Sci. 45: 389-399.
- PATTERSON, R. 1984. Flavonoid uniformity in diploid species of Hawaiian *Scaevola* (Goodeniaceae). Syst. Bot. 9: 263-265.
- PHILIPPI, R. A. 1856. Bemerkungen uber die flora del Insel Juan Fernández. Bot. Zeitung (Berlin) 14:

- 641-648.
- SANDERS, R. W., T. F. STUESSY, C. MARTICORENA and M. SILVA O. 1987. Phytogeography and evolution of *Dendroseris* and *Robinsonia*, tree-Compositae of the Juan Fernández Islands. *Opera Bot.* 92:195-215.
- SANDERS, R. W., T. F. STUESSY and R. RODRIGUEZ. 1983. Chromosome numbers from the flora of the Juan Fernández Islands. *Amer. J. Bot.* 70: 799-810.
- SCHINDLER, A. K. 1905. Halorrhagaceae. *In*: A. Engler (ed.), *Das Pflanzenreich* 4: 104-128. Wilhelm Engelmann, Berlin.
- SKOTTSBERG, C. 1921. The phanerogams of the Juan Fernández Islands. *The Natural History of Juan Fernández and Easter Island* 2: 95-240, 11 lám.
- SKOTTSBERG, C. 1951. A supplement to the pteridophytes and phanerogams of the Juan Fernández and Easter Island. *The Natural History of Juan Fernandez and Easter Island* 2: 763-792, 3 lám.
- SKOTTSBERG, C. 1956. Derivation of the flora and fauna of Juan Fernández and Easter Island. *The Natural History of Juan Fernández and Easter Island* 1: 193-439, 1 mapa.
- SNOW, R. 1963. Alcoholic hydrochloric acid-carmines as a stain for chromosomes in squash preparations. *Stain Technol.* 38: 9-13.
- SPOONER, D. M., T. F. STUESSY, D. J. CRAWFORD and M. SILVA O. 1987. Chromosome numbers from the flora of the Juan Fernández Islands II. *Rhodora* 89: 351-356.
- ST. JOHN, H. 1946. Endemism in the Hawaiian flora, and revision of the Hawaiian species of *Gunnera* (Haloragidaceae). *Hawaiian plant studies II. Proc. Calif. Acad. Sci.* 25: 377-420.
- ST. JOHN, H. 1957. *Gunnera magnifica*, a new species from the Andes of Colombia. *Svensk Bot. Tidskr.* 51: 521-528.
- STUESSY, T. F., K. A. FOLAND, J. F. SUTTER, R. W. SANDERS and M. SILVA. 1984. Botanical and geological significance of potassium-argon dates from the Juan Fernández Islands. *Science* 225: 49-51.
- STUESSY, T. F., D. J. CRAWFORD and C. MARTICORENA. 1990. Patterns of phylogeny in the endemic vascular flora of the Juan Fernández Islands, Chile. *Syst. Bot.* 15: 338-346.
- SUN, B. -Y., T. F. STUESSY and D. J. CRAWFORD. 1990. Chromosome counts from the flora of the Juan Fernández Islands, Chile. III. *Pacific Sci.* 44: 258-264.
- WELLER, M. W. 1980. *The Island Waterfowl*. Iowa State University Press, Ames, Iowa.
- WILKINS, C. K. and B. A. BOHM. 1976. Chemotaxonomic studies in the Saxifragaceae sensu lato 4. Flavonoids of *Heuchera micrantha* var. *diversifolia*. *Canad. J. Bot.* 54: 2133-2140.
- WITTER, M. S. and G. D. CARR. 1988. Adaptive radiation and genetic differentiation in the Hawaiian Silversword alliance (Compositae: Madiinae). *Evolution* 42: 1278-1287.

Fecha Publicación: Junio 1993